Center for Skeletal Research

MGH Endocrine Unit

Imaging & Biomechanical Testing Core
Core Director: Mary Bouxsein, Ph.D
Core Manager: Daniel Brooks, MSc
Core Technician: Michael Armanini, MSc
Email: microCTcore@partners.org

Preparation of samples for µCT scanning

µCT scanning is a non-destructive process and can be performed prior to other types of analyses such as histology and mechanical testing. µCT can be performed on either fixed or fresh-frozen samples depending on the type of post-µCT analysis that will be performed on the samples. In general, the bone that you are interested in analyzing, should be isolated from the adjacent bones and cleared of as much tissue as possible without damaging the bone (please refer to the special instructions on the second page for the preparation of commonly scanned mouse and rat bones). For best image quality, we must scan samples in some sort of liquid medium, so please submit your samples in the liquid that you would prefer that we use for scanning. Please follow the instructions below based on the future analysis that you plan to perform on your samples.

Samples that will subsequently be sent to histology

The samples should be prepared based on the fixation protocols available from the Histology & Histomorphometry Core website. We can scan samples in most liquids (PBS, ethanol, formalin, and most other liquids), so you can submit your samples in the storage liquid recommended in your histology protocol. Once the µCT analysis is completed, you can submit the bones to the histology core for processing.

Note about decalcification:

As a general rule, do not decalcify your samples prior to submitting them for µCT scanning, as this will remove the mineral and prevent imaging of the boney structures. If your histology protocol required decalcification of the samples, you can perform the decalcification step after the µCT scanning had been completed. There may be some cases where you may want to decalcify the samples prior to µCT scanning. Two examples of samples that may need to be decalcified prior to µCT scanning are samples that contain contrast agent filled vasculature and samples that have been stained with a radiopaque stain (i.e. osmium tetroxide staining of marrow fat). Please consult us prior to decalcifying your samples if you believe that decalcification may be necessary.

Samples that will subsequently be mechanically tested

Samples that will be mechanically tested should be fresh-frozen soon after they are harvested using the following procedure:

1) Harvest the samples. A protocol for harvesting various mouse bones is available (please email us if you would like us to send you this protocol).
2) Wrap the bone in PBS soaked gauze and insert it in a labeled tube (1.5mL or 2mL microcentrifuge tubes work well for mouse long bones and vertebrae).
3) Store the samples at -20°C. The Imaging & Biomechanical Core has a -20°C freezer for storing your samples while we are performing your study. Please note that you should not store your samples at -80°C prior to mechanical testing because it may cause your samples to become brittle.

Note: Collection of bones for mechanical testing is not extremely time critical. The most important thing is to ensure that the bones stay hydrated. In most cases it should be fine for you to collect all of your samples and then transfer them to the freezer. If you are performing a prolonged harvest over the course of many hours, it is recommended that you store your samples on ice once you harvest them, and then transfer them to the freezer.

Samples that will be stained (osmium tetroxide) for marrow fat quantification

Osmium tetroxide is radiopaque and can be used to stain marrow fat in mouse long bone. The stained marrow fat can subsequently be scanned and quantified using µCT. Osmium tetroxide staining requires that bones be decalcified prior to staining. We ask that you fix (but not decalcify) your bones using the *Protocol for preparing bone for osmium tetroxide staining* available at the Imaging and Biomechanical Testing Core website and then submit them to us so that we can scan the calcified bones to measure bone architecture. After we perform the scans of the calcified bones, we will decalcify the samples and perform the osmium tetroxide staining. Note: Do not place bone in solvents (ethanol, acetone,…) if you plan on performing marrow fat analysis. Doing so will solubilize the marrow fat and preclude the samples from being used for marrow fat analysis.

Special instructions for commonly scanned mouse and rat bones

Please refer to the *Protocol for Harvesting Mouse Long Bones and Vertebrae* on the Imaging & Biomechanical Core website for specific dissection instructions. The following are some guidelines for submission of some commonly scanned rodent bones:

Femurs:

- The femur should be isolated from the pelvis and tibia.
- Be careful to leave the femoral head and distal femur intact, as these are used as reference points when positioning the scans. The distal end of the femur can easily separate at the growth plate if you are not careful.
- If the femoral head is missing or the distal femur separated at the growth plate, it may still be possible to scan and analyze the sample (the region of interest for the trabecular analysis is just above the distal growth plate and the cortical analysis is performed at the mid-diaphysis). If the distal femur breaks above the growth plate, it will not be possible to perform the trabecular analysis.

Tibiae:

- Isolate the tibia from the femur and the foot, but leave the fibula intact if possible (the tibiofibular joint is used as a reference point when positioning scans). If the fibula breaks off (and it often does) it will still be possible to analyze the sample.
- Trabecular analysis is typically performed in a region just distal to the proximal growth plate.
- Cortical analysis is typically performed on the cortex above the tibiofibular joint.

Vertebrae:

- If possible, isolate the vertebra that you are interested in analyzing (the L5 vertebra is the one that is typically analyzed). If you are not confident that you can consistently isolate the same vertebrae you can submit the lumbar spine with the pelvis attached and we will pick up the correct vertebra in the scout-view when we set up the scan (there will be an additional fee for this because it takes longer to set up and scan samples using this method).
- It can be difficult to clean all of the soft tissue off of the vertebrae. For the purpose of μCT it is okay to leave soft tissue on the bone.
- Trabecular bone analysis is typically performed in the vertebral body. It is okay if the vertebral processes are broken.